**Case 30: Congenital Asplenia**

**Summary:** Adaptive immune response occurs mainly in the peripheral lymphoid tissues (nodes, GALT, spleen). Pathogens and their secreted Ags are trapped in these tissues and presented to the naïve lymphocytes that constantly pass through. Pathogens that enter through the skin or lungs drain to regional lymph nodes, enter through the GI are collected in the GALT, enter the bloodstream stimulate immune response in the spleen. Lymphocytes arise from stem cells in the bone marrow and differentiate in central lymphoid organs (bone marrow or thymus). Migrate from these tissues trhough the blood to periperhal lymphoid tissues (nodes, spleen, mucosal lymphoid tissues such as tonsils, Peyer’s patches, appendix) where they are activated by antigen. Lymphatics drain extracellular fluid as lymph through the LN and into the thoracic duct, which returns the lymph to the bloodstream by emptying into the L subclavian vein. Lymphocytes that circulate in the bloodstream enter the peripheral lymphoid organs and are eventually carried by lymph to the thoracic duct where they reenter the blood stream.

Spleen is organized to accomplish 2 functions. In addition to being a peripheral lymphoid organ, it acts as a filter of the blood to remove aged or abnormal RBCs and other extraneous particles that may enter the bloodstream, including pathogens. In absence of a functioning spleen, these aged and abnormal RBCs can be seen in peripheral blood smear in the form of pitted RBCs and Howell-Jolly bodies (inclusions formed from nuclear remnants in RBCs that are usually removed from the spleen).

The lymphoid function of the spleen is performed in the white pulp and the filtration function (RBC destruction) by the red pulp. Many microbes are directly recognized and engulfed by phagocytes in the red pulp. Others aren’t removed efficienctly untile they are coated by antibodies generated in the white pulp. The white pulp consists of a follicle and T cells areas that are surrounded by the perifollicular zone. The follicular arteriole emerges in the periarteriolar lymphoid sheath (PALS) of T cells, traverses the follicle, goes through the marginal zone, and opens into the perifolicular zone. Immune response (measured by antibody formation) can be detected in the white pulp about 4 days after pathogen exposure. The clearance of Ab and complement coated bacteria or viruses by the phagocytic cells of the red pulp is very rapid. Rapid clearance from the blood is important because it prevents bacteria from disseminiating and causing meningitis, kidney infections (pyelonephritis), pneumonia, or infection at other distant sites.

Bacteria enter the bloodstream all the time (brushing teeth, local infections such as skin and otitis media). Normally, these bacteria are disposed of efficiently by the spleen. When the spleen isn’t present, serious and possibly fatal infections occur. Significant impairment of splenic function can be congenital (where the spleen is absent or dysfunctional at birth), or acquired as result of conditions that damage the spleen (such as trauma or sickle cell anemia).

Congenital asplenia is further divided into 2 categories. Syndromic asplenia (more common) is where lack slenic tissue is part of a more complex genetic syndrome affecting other systems as well, including heart defects and herteotaxis (malrformations arise as a result of lateralization defects of organs in the throax and abdomen). Genes such as ZIC3, LEFTYA, CRYPTIC, ACVR2B, and CFC1 have been mutated in these syndromes, and all have important roles in directing lateralization. Isolated congential asplenia is the second category as is when lack of splenic tissue is the only abnormality (small number of cases). Most cases are diagnosed after episodes of overwhelming pnemococcal infections either post mortem or while screening family members. The genetic defect for the cases has not been identified. Most cases follow and autosomal dominant pattern although some familes have been reported with an autosomal recessive or X linked pattern of inheritance.

Surgical removal of the spleen is common. The capsule of the spleen may rupture from trauma (automobile accident). In these cases, the spleen must be removed quickly because of blood loss into the abdominal cavity. The spleen may be removed for therapeautic reasons in certain autoimmune diseases or because of malignancy. After removal, patients, particularly kids, are susceptible to bloodstream infections by microbes against which they have no antibodies. Microbes against which the host has antibodies are removed quickly from the bloodstream by the liver where Kupffer ells completment the role of the red pulp of the spleen. Antibodies against the encapsultaed bacteria that commonly cause bloodstream infections persist for a very long time in the bloodstream of exposed individuals even in absnece of a spleen. Afults who already have antibodies aginst these pathogens are therefore much less vulnerable to bactermia than kids who have no yet developed such antibodies. Effective vaccines against both s. peumo and h. influenae type b have been developed and are part of routine vaccinations, thus protecting aspenic kids from some severe infections. Neverthelss, specific precations, including prophylactic antiboiotics treatment (taken at low doses daily, higher doses with any dental work or invasive surgical procedures), are recommended for patients.

Major consequence of spleen absence is susceptibility to bacteremia, usually caused by encapsulated bacteria such as strep pneumo or h. influenzae. Susceptibility is caused by a failure of the immune response to these common extracellular bacteria when they enter the bloodstream.

**Case:** parents distantly related, 10 mo old girl developed cold lasting 2 weeks, became sleepy and had a high fever, convulsive movements of her extremities and died on the way to the hospital, post mortem cultures of blood, throat, and CSF grew h. influenzae type b, at autopsy found to have no spleen

3 yr old sister also had a fever, earache, elevated WBC count, nose, throat, and blood cultures grew H. influenzae type b, treated later in the year for otitis media, pneumonia, and mastoiditis

5 yr old brother had 2 previous episodes of strep pneumo meningitis, also had pneumonia episode

2 other siblings were in good health

All kids received routine immunizations with DtaP, serum agglutination tests were normal (Ab protects cell by binding to the toxin), all kids given suspension of sheep red ccells IV, brother and sister didn’t have increase in titer but 8 yr old normal sister had increase in hamgglutinating titer 2-4 weeks after immunization against sheep red blood cells

Kids and parents injected IV with radioactive colloidal gold which is taken up by reticuloendothelial cells of the liver and spleen within 15 minutes of injection, scintillation counter scans the abdomen for radioactive gold, pattern of scintillation revealed that the brother and sister had no spleens (no small mass on the right)

This family is unusal in that 3 or the first 4 kids were born without a spleen. After these events, the family have 3 more kids. 2/3 kids had no spleen and the other had a normal spleen. Shows that this was inherited as autosomal recessive. Parents are normal but each carries the recessive genes. They are consanguineous (setting where autosomal recessive conditions are more frequently encountered). Each pregnancy provides a ¼ chance that the fetus will inherit the abnormal gene from both parents. If the sister with no spleen marries a normal man, all her kids will be heterozygous but will have normal spleens.

Normal responses to typhoid vaccine but not sheep RBCs because the typhoid vaccine was given subQ and response was mounted in a regional LN. The sheep RBC was given IV and in the absence of a spleen failued to enter any peripheral lymph tissue where an immune repsonse to them could occur.

**Describe the adaptive immune response as occurring mainly in secondary lymphoid tissues (lymph nodes. mucosal associated lymphoid tissue, and spleen)**

**Explain that the spleen is the main trap for microbes and particulate antigens entering the blood. (In contrast, the main traps for antigens entering via the skin are the draining lymph nodes and, for those entering via through the gut, the gut-associated lymphoid tissue.)**

**Explain that bacteria are filtered out of the blood predominantly in the red pulp of the spleen and this can lead to an immune response, such as antibody production, that takes place in the white pulp.**

**Explain that the spleen filters out blood borne microbes better when they are coated with opsonins such as antibody (and complement which will learnt about later). Be able to explain that the Fc of IgG antibodies bound to particulate antigens produces this opsonizing effect by acting as a flag for spleen macrophages that have Fc receptors.**

**Explain that lack of a spleen in a child greatly increases susceptibility to several bacteria that cause bacteremia (bacterial infection of the blood) and that the increased residence in the blood is due to a greatly delayed antibody response giving rise to opsonizing antibodies.**

**Explain that adults who lose their spleen tend to be little affected in susceptibility to bacterial infections as they have already developed opsonizing antibodies allowing attack on bacteria by neutrophils and macrophages once they have migrated out of the blood.**

**Explain that immunizations are both advised and effective in reducing the risk of severe infection by *Haemophilus influenzae* type band *Streptococcus pneumoniae* in persons with asplenia. Explain that the antigen in a vaccine is not processed by the spleen but is given s.c. or i.m. and so is processed by draining lymph nodes that retain normal activity in asplenic persons.**

**Explain that prophylaxis with antibiotics given daily is often advised for asplenic patients and pretreatment with antibiotics is very important at times when large numbers of bacteria may be expected to enter the blood (such as when getting dental work)**

**Explain how scans for radioactive colloidal gold can be used to detect the presence or absence of a spleen.**

IV injection of radioactive colloidal gold. Scintillation scan of abdomen to see which reticuloendothelial cells (liver and spleen) take up the gold within 15 minutes. Large mass on the left is liver, small mass on the right would be the spleen.

**Case 31: Hereditary Angioedema (HANE or HAE)**

**Summary:** Complement is a system of plasma proteins that participate in a cascade to generate active components that allow pathogens and immune complexes to be destroyed and elminiated from the body. Part of the innate system and is also activated via antibodies produced during the adaptive response. Complement activation is usually confined to the surface of pathogens or circlating complexes of Ab bound to Ag.

Normally activated by 1 of 3 routes: classical pathway is triggered by Ag:Ab complexes or Ab bound to pathogen surface, lectin pathway is triggered by mannose-binding lectin and ficolins, and the alternative pathway is where complement is activated spontaenously on the surface of some bacteria. Early pathways involve proteolytic cleavage events leading to generaiton of a serine protease convertase that cleaves C3 and initiates effector actions of complement. The C3 convertases of the pathways were different by evolutinoarily homologous enzymes.

The principle molecule and focal point of activation for all of the systems is C3b cleavage fragment from C3. If active C3b (or homologus but less potente C4b) accidentally becomes bound to a host cell surface instead of a pathogen, the cell may be destroyed. This is usually prevented by the rapid hydrolysis of active C2b and C4b if they don’t bind immediately to the surface where they were generated. Protection against inapropriate activation of complement is protected via regulatory proteins.

The most potent inhibitor of the classical pathway is C1 inhibitor (C1INH), part of the serine protease inhibitor family (serpins) which together constitiue 20% of all plasma proteins. In addition to being the sole inhibitor of C1, C1INH also contributes to the regulation of serine proteases of the clotting system and kinin system, which is activated by blood vessel injury and some bacterial toxins. The main product of the kinin system is bradykinin, which causes vasodilation and increased capillary permeability.

C1INH intervenes in the first step of complement, when C1 binds to Ig molecules on the pathogen surface or IC’s. Binding of 2 or more of the globular hears of C1q is required to trigger sequential activation of the 2 associated serine proteases, C1r and C1s. C1ING inhibits both of these proteases by presenting them with a bait site in the form of an arginine bond that they cleave. When C1r and C1s attack the bait site, they covalently bind C1INH and dissociate from C1q. This causes C1ING to limit the time during which Ab bound C1 can cleave C4 and C2 to generate the classical C3 convertase (C4B2b).

Activation of C1 also occurs spontaneously at low levels without binding to Ag:Ab complex and can be triggered further by plasmin (protease of the clotting system) which is normally inhibited by C1ING. In absence of C1ING, active components of complement and brdykinin are produced and are seen in the disease hereditary angioedema, caused by genetic deficiency of C1INH.

Patients are subject to recurrent episodes of circumscribed swelling of the skin, intestine and airway. Attacks of subQ or mucosal swelling most commonly affect the extremities, but can also involve the face, trunk, genitals, lips, tongue, or larynx. Cutaneous attacks cause temporary disfigurement but aren’t dangerous. When swelling occurs in the intestine, it causes severe abdominal pain and obstructs the intestine so that patients vomits. When the colon is affected, watery diarrhea may occur. Swelling in the larynx is the most dangerous symptoms because the patient can rapdily choke to death. Attacks usually don’t involve itching or hives (useful to differentiate from allergic angioedema). A serpiginous or linear and wavy rash is sometimes seen before onset of swelling. Epidoes may be triggered by trauma, menstraul periods, excessiv exercise, exposure to temperature extremes, mental stress, and some medications (angiotension-converting enzyme inhibitors and oral contraceptives).

Not an allergic disease and attacks aren’t mediated by histamine. Attacks are associated with activation of 4 serine proteases, which are normally inhibited by C1INH. Factor XII directly or indirectly activates the other 3 proteases. Factor XII is normally activated by blood vessel injury and initiates the kinin cascade, activating kallikrein, which generated the vasoactive peptide bradykinin. Factor XII also indirectly activates plasminin, which actiavates C1 itself. C1 cleaves C2 and plasmin cleaves C2b to generate the vasoactive fragment C2 kinin. In HAE patients, uninhibited activation of Factor XII leads to activation of kallikrein and plasmin. Kallikrein catalyzes the formation of bradykinin and palsmin produces C2 kinin. Bradykinin is the main mediator responsible for attacks by causing vasodilation and increasing the permeability of the postcapillary venules by causing contraction of endothelial cells to create gaps in the blood vessel wall. This is resposible for the edema (movement of fluid from vascular space into the gut causes symptoms of dehydration as the vascular volume contracts).

Patients aren’t unduly susceptible to infections because the alternative pathway of complement is intact and although the classical pathway is affected by deficiencies of C2 and C4, these are compensated for by the potent ampliication step from the alternative pathway.

Inherited as autosomal dominant. Patients with HAE synthesize about 37-40% of the normal amount of C1INH and C1INH catabolism is 50% greater than in normal controls.

**Case:** 17 yr old boy, attacks of severe abdominal pain with frequent sharp spasms, began to vomit every 5 minutes, no point tenderness to indicate appendicitirs, dry mucous membranes, elevated RBC count indicated dehydration, exploratory ab surgery revealed a moderately sweollen and pale jejunum but no other abdnomalities, had episodes of ab pain since 14 yrs old, his mother, maternal grandma, maternal uncle, and sister also had recurrent episodes of severe abdominal pain, as newborn he was prone to severe colic, at age 4 a bump on his head led to abnormal swelling, at 7 yrs a injury with a baseball bat caused his entire forearm to swell twice its normal size (swelling wasn’t painful, red, or itchy, dissapeared 2 days later), at age 14 becan to complain of ab pain every few months sometimes accompanied by vomiting and rarely by clear, watery diarrhea, complement levels show that C1INH levels were 16% of normal, C4 was markedly decreased, C3 was normal

C4 was decreased because it was rapidly cleaved by active C1, the only other component that should be decreased is C2 which is also cleaved by C1. C1 plays no part of the alternative pathay so this shouldn’t be affected. The terminal components aren’t affected either. The unregulated activation of the early complement components don’t lead to formation of the C3/C5 convertases, so the terminal components aren’t abnormally activated. The depletion of the early components of the classical pathway doesn’t affect the response to the normal activation of complement by bound antibody because the amplification of the response through the alternative pathway compensates for the deficiency in C4 and C2.

**Treatment:** focuses on preventing attacks or on resolving acute episodes, purified or recombinant C1INH is effective in both settings, kallikrein inhibitor and bradykinin receptor antagonist have also been developed, Winstrol (stanozolol, anabolic androgen, not a good drug for women because it causes wiehgt gain, acne, and sometimes amenorrhea)

**Describe the classical pathway for activation of complement.**

C1q binds to IgM (staple conformation) or to at least 2 IgG molecules on the pathogen surface or IC via the globular heads to the Fc region. This activates the associated C1r, which then cleaves C1s proenzyme, which then initiates that classical complement cascade. C3b binds covalently to pathogens and opsonizes bacteria, enabling phagocytes to internalize them and also binds to ICs to permit their clearance. C3a and C5a are produced an are mediators of inflammation and recruit phagocytes. C5b triggers the late events in which terminal components assemble to form the MAC and damage pathogen membranes leading to their lysis. It is now known that C1q can activate the pathway by binding directly to pathogen surfaces.

**Explain that activated forms of C1r and C1s are proteinases that are inactivated by binding to a serine protease inhibitor named C1INH**

Active C1 is inactivated by C1INH by binding covalently to C1r and C1s causing them to dissociate from the complex. Since there are 2 C1r and 2 C1s molecules for each complex, it takes 4 molecules of C1INH to inactivate the C1r and C1s.

**Explain that C1INH inactivates other proteinases including proteinases generated in the clotting and kinin systems**

**Explain that lack of C1INH causes hereditary angioedema (old name = hereditary angioneurotic edema)**

**Describe how HANE is associated with abnormal swelling of tissues (edema) and occurs especially after trauma.**

**Describe how the poor control of activated C1 leads to generation of excessive cleavage of C2 releasing a small peptide that is further cleaved to produce C2 kinin. Explain how C2 kinin and bradykinin then cause endothelial cell contraction in post capillary capillary venules and so allow fluid release into the tissues with the formation of edema.**

**Explain that in HANE, activation of C2 and C4 occurs in the plasma and, unlike their activity on more protected sites on cell surfaces, the larger fragments are inactivated rapidly so they do not form an effective C3 convertase. Thus mast cell degranulation associated by anaphylatoxins C3a and C5a generation is not seen. Because histamine is not produced, the swelling in HANE does not itch.**

Histamine release on complement activation is caused by C3a and C5a (main chemokine). Both are generated by convertases. In HAE, the C4b and C2 fragments are generated free in plasma. They are rapidly inactivated if it doesn’t bind immediately to a cell surface, so because the concentrations of the fragments are low, no convertases are formed and C3 and C5 aren’t cleaved. The edema isn’t caused by the inflammatory mediators in late complement activation, but by C2b generated during early events and by bradykinin generated by uninhibited activation of the kinin system.

**Describe the finding that patients with HANE are resistant to infections, presumably because of a fully functional alternative pathway….**

**Describe the autosomal dominant inheritance of HANE.**

**Describe the use of androgen therapy to induce increases in plasma C1INH concentration and the disadvantage of using this treatment in women.**

**Describe how there is a recently approved FDA drug, known as CINRYZE®, which has been in use for many years in Europe.. This drug is purified human C1INH and is now the drug of choice.**

**Case 32: Factor I Deficiency**

**Summary:** Pathogens coated with complement are more efficiently phagocytosed by macrophages and bacteria coated with complement can also be directly destroyed by complement-mediated lysis. The alternative pathway is important in innate/nonadaptive immunity. It can be activated in absence of antibody, although even low titers of IgM Ab against an infecting microbes will greatly amplify comlement activation. C3 is the starting point of the alternative pathway. One of the most abundant globulins in the blood and contains a highly reactive thioester bond. Is continuously being cleaved by spontaneous hydrolysis at a fairly low rate to form intermediateC3 in the plasma via tickover. iC3b associated with Bb from fB to form a short-lived soluble C3 convertase. This proteinase cleaves many C3 molecules to produced a C3b fragment with the thioester bond that can covalently bind to the hydroxyl group of a serine or threonine in a protein of the hydroxyl of a sugar on the microbe surface. If C3b doesn’t bind, the thioester bond is spontaneously hydrolyzed and C3b is inactivated.

Binding of C3b to microbe surface stimulates the cleaves of more C3 molecules. fB binds to C3b on the surface and is then bound by preexisting blood proteinase fD leavaing Bb bound to C3b. This forms an active serine protease known as C3 convertase which can cleave C3 to make more C3b and C3a. C3b bound to the surface acts as an opsonin by binding to a CR3 on phagocytes to facilitate ingestion of C3b-coated particles. Before C3b can act as a ligand for CR3, it must undergo further cleavage to iC3b via blood serine protease fI in conjunction with blood protein fH (fH displaces B from C3bBb complex and factor I then cleaves C3b to produce iC3b). iC3b acts via CR3 to activate neutrophils and macrophages in the absence of antibody. Cleavage of C3b by fI also inhibits C3 convertase activity of the C3b complex, thus ensuring that supplies of C3 don’t become depleted. On host cell surfaces, CR1 can bind to C3b and serve as a cofactor instead of fH.

Genetic deficiency of fI impacts the innate immunity in killing against common extracellular bacteria that cause pyogenic infections. The lack of fI means that the alternative pathway C3 convertase is uninhibited and consumption of C3 is greatly accelerated, leading to C3 depletion. The lack of C3 and the nonproduction of iC3b leads to defective opsonization, which is the main means of removing and destroying these bacteria. Thus, fI deficiency like C3 deficiency results in greatly increased susceptibility to infections with pyogenic bacteria. Similar clinical findings to X linked agammaglobulinemia (failure to opsonize leads to frequentpyogenic infections).

Gene encoded on chromosome 4. Autosomal recessive inheritance. If heterozygous for the defect they produce roughly half the normal amounts of fI which are sufficient to prevent any clinical symptoms.

Hives were caused by the constant cleaved of C3 to C3a and C3b. C3a binds to mast cells and causes release of histamine leading to hives. Didn’t have hives all the time probably because he had tacyphylaxis (end organ unresponsiveness to histmine). Only when histamine released was increased by alochol of sudden changes in ambient body temperature, did the symptoms appear.

Meningooccemia is symptomatic of deficency in components of the alternative pathway of complement (both neisseria). Patients have died from septic shock within 20 min of onset of symptoms. Patients with genetic defects in alternative pathway or terminal components sustain overwhelming and repeated infection with Neisseria. Deficiencyes in alternative pathway components fD and properdin were also disovered in patients with recurrentmeningococcemia. Highligh important of bactericidal action of complement in controling septicemia due to Neisseria.

Complement fI and fH are essential regulators of complement activation. While complete deficiency in either one of these factors results in increased occurrence of pyogenic infections, heterozygous mutations of fH or fI have been identified in patients with atypical hemolytic uremic syndrome (glomerular thrombotic microangipathy). fH mutations may also cause membranopropliferative glomerulonephritis with dense deposits in glomeruli and age related macular degernation. Disease are characterized by inability of mutated fH to control C3 activation at the cell surface, whereas regulation of C3 activation in the plasma is normal.

**Summary:** 25 yr old with pneumonia, 28 total hospital admissions including middle ear infections, mastoiditis, pyogenic (pus forming bacteria) were cultured including s. aureus, proteus vulgaris, pseudomonas aeruginosa, age 3 had a tonsillectomy and adenoidectomy because of enlargement and chronic infection of nasopharyngeal lymphoid tissue, at age 6 had scarlet fever, lower left lobe pneumonia with h. influenzae, abscess in grion, acute sinusitis, posterior ear abscess due to corynebacterium, skin abscesses with accompanying bloodstream septicemia due to beta hemolytic strep and septicidemic due to n. meningitidis, PE showed he was obese but otherwise developed normally, hearing was poor due to recurrent ear infections and mastoiditis, developed hives after drinking alcohol or after taking a bath or shower, normal urine analysis, normal hematocrit, WBC, platelets, blood clotting, RBC gave strong agglutination with Ab to C3 but none with Ab to IgG or IgM, normal IgG, IgA, IgM, responded to tetanus toxoid, antibody titer rised with hemagglutin, positive delayed type skin reaction to mumps and monillia antigens, serum C3 was low, C3b was in plasma, fB was undectable, all other complement levels were normal, serums didn’t kill smooth strain of salmonella enterica Newport even after addition of C3 to the serum, injected with C3 labeled with radioactive I tracer, showed that C3 synthesis was normal but it was being broken down very quickly (increased rate of dissapearance, fB would also be rapidly destroyed due to increased binding of fB to C3b and cleavage by fD), test of serum with Ab against fI showed that he lacked fI, no family history of recurrent bacterial infections but showed reduced levels of fI in both parents and several siblings

RBC agglutinated against C3 because he is producing large amounts of C3b, which binds to CR1 on RBCs and leads to their agglutination by anti-C3.

Clinical course improved with age and now had fewer infections. Adaptive immunity against these common bacteria becomes stronger and he has come to rely less on innate immune mechanisms for his protection. Bacteria coated with Ab can be phaogyctosed independelt of complement via Fc receptors on phagocytes.

Give IV fI: serum C3 and fB levels rise to normal and C3b dissapeared from serum

**Describe the alternative pathway for activation of complement, including the interactions of surface bound C3b with factor B, and the action of factor D on this complex to generate C3b,Bb (which is itself a C3 convertase).**

**Explain that C3b bound on host cell surfaces is immediately cleaved to iC3b by factor I, using factor H, C4bp, CD46 (MCP), or CR1 as a cofactor. The inactivation of C3b by factor I (iC3b) inhibits the formation of convertases (i.e. iC3b cannot bind factor B). This inhibits the activation and amplification of the cascade on host cells. The amount of iC3b on host cells ends up being insignificant.**

**Describe that iC3b (which is in high amounts on pathogen surfaces where complement has efficiently activated and amplified) is an opsonin and binds to CR3 (and CR4) on phagocytes, leading to enhanced phagocytosis.**

**Explain that patients with a deficiency of factor I fail to inactivate C3b, leading to enhanced formation of more convertases (C3b,Bb), which in turn leads to depletion of C3 as well as factor B.**

**Explain that patients with a deficiency in factor H (essential cofactor for factor I and important decay acceleration function) have identical clinical parameters as factor I deficiency.**

**Explain that patients who lack of H, I or C3 have a greatly increased susceptibility to pyogenic infections (similar to those with agammaglobulinemia) and also are susceptible to disseminated Neisseria infection. This latter is most likely due to the failure to generate a C5 convertase on the bacterial surface and formation of the membrane attack complex (MAC). (Remember that C5 convertases contain C3, thus depleting C3 affects C5 convertase as well).**

**Deficiency in fH can lead to same clinical picture and lab results. Because fH is needed for cleavage of C3b by fI in the blood, the clinical symptoms would be indistingushable.**

**Explain that the C3a generated by cleavage of C3 can cause mast cell degranulation. When extensive degranulation is seen, hives develop.**

**Case 33: Complement C8 deficiency**

**Summary:** Complement is an effector mechanism of both innate and adaptive immunity that tags pathogens for destruction. Terminal components (C5-C9) on the surface of the pathogen form a protein complex that makes pores in the cell membrane leading to cell lysis and death. Initiated by C5 binding to cell surface and being cleaved by C5 convertase. This releases C5a (chemotactic peptide from the alpha heavy chain) and allows C5b to bind C6. The C5b6 complex binds C7, resulting in exposure of a hydrophobic site on C7 that enables the complex to sink partway into the cell membrane. C8 is composed of 3 chains. The C8g chain binds to the C5a67 complex and enables the hydrophobic portion of C8 to embed itself in the cell membrane. This induces the polymerization of 10-16 molecules of C9 to make a MAC which forms a pore in the membrane. Through this channel, Na and water enter the cell causing it to swell and burst (lysis). The cell surface protein CD59 found on most mammalian cell membranes inhibits action of C8 and C9 to prevent the formation of MAC on the cells of the body.

Neisseria bacteria have a thick carb capsule. The hydrophobic components of the MAC can’t penetrate the polysaccharides of the capsule. The bacteria are temporariliy vulnerable to killing when they divide. At this time the membrane is exposed and is vulnerable to attack by MAC. The association between geneic deficiencies of MAC and neisserial infections illustrates that an important host defnse against these infections is killing the extracellular bacteria via lysis. Neisseria that escape killing entery a variety of cell types and establish intracellular infections.

Humans with deficiency in C5-C9 have increased susceptibility to systemically invasive infection with Neisseria. High frequency of particular complement deficiencies in certain populations. 1/40 Japanese are gertozygous for C9 deficiency. Deficency of C8 in Caucasian populations is caused by mutations in the gene for the beta chain. C8 defiency in African Americans is commonly due to mutations in the gene for the alpha chain. More than 90% of mutations in C8b are due to C-T transistions in the C8B gene.

**Case:** College student developed cough and diarrhea, tired and achy, neck stiff and somewhat confused, low BP, fast pulsef and RR, elevated temp, petechial rash on chest, palate, and extremities, red throat with moderate enlargement of the tonsils, blood cultures grew n. meningitidis serogroup C (treated with cephtriaxone), CSF was sterile but had WBCs, slightly low hematocrit, high neutrophils, had meningococcal meningiti once before with positive CSF culture from serogroup Y, CH50 assay (complement mediated hemolysis) was 0

Family history: 3 of her sisters had CH50 of 0 and one of them had meningiococcal meningitis before, older sister had normal CH50, mom had half normal CH50, double diffusion assays in agar showed lack of C8, as did her 3 sisters, mother had half normal level of C8

Probably got C8b mutation from Caucasian dad. Filipino mom was heterozygous for another type of mutation in the C8B gene not found in Caucasians. Patient was therefore compound heterozygous defieincy for the C8B gene.

**Treatment:** prophylaxis with tetravalent mingococcal vaccine

**Describe the roles of the terminal components of the complement pathway (Fig 6.1).**

C5a: small peptide mediator of inflammation

C5b: initaites assembly of MAC

C6: binds C5b, forms acceptor for C7

C7: binds C5bC6, ampiphilic complex inserts into bilayer

C8: binds C5b67, initiates C9 polymerization

C9: polymerizaes to C5b678 to form a membrane spanning channel to lyse the cell

**Describe the role of the C5 convertase in initiating the steps leading to formation of the membrane attack complex.**

C5b triggers assembly of C6, C7, C8. C7 and C8 undergo conformational changes that expose hydrophobic domains that insert into the membrane. Causes polymerization of C9 against with exposure of the hydrophobic site. Forms pore

**Explain that deficiency in production of a membrane attack complex (deficiency in C5-C9) leads to susceptibility for infections by *Neisseria* species, and that this susceptibility also occurs from deficiencies in properdin and factor D (alternative pathway).**

**Describe how the quantity of hemolytic complement is measured from the CH50 values, which can be used a screening test for complement deficiencies.**

Quantity of complement required for 50% lysis of 5 x 108 optimally sensitized sheep RBCs in 1 hr at 37C (sensitized with rabbit Ab against the sheep cells). The complement titer is expressed as the number of CH50 contained in 1 ml of undilated serum from the patient. This is determined by plotting log y (1-y) vs. serum added and the plot is linear near y/(1-y)=1 (50% lysis) where y is the percentage of lysis. Because this assay measures the functional integrity of all the complement componenets comprising the classical patway, it is the single best screening test when a eneal diagnosis of complement complement deficiency is being considered.

**Explain that deficiencies in C1q, C1r or C1s, (classical pathway), and C4 are associated with development of severe immune complex disease (antigen:antibody compexes) which are frequently fatal. This appears to result from a lack of C4b, which is important for attaching immune complexes to CR1 on red cells for efficient immune complex removal by the liver.**

C1q, C1r, C1s or C4 patients sustain persistent and severe IC diseases such as glomerulonephritis which can be fatal. All the C1 components are required for formation of C4b and if C4b is part of an IC, it will bind to CR1. IC bound to the surface of RBCs are transported to the liver and spleen where C4b is converted by fI to iC4b to facilitate uptake of ICs by phagocytes via CR3 and their destruction. In the absence of C4b, ICs are less efficiently attached to RBCs and are less efficiently cleared from the blood.

C2 deficient patients sustain only minor forms of IC disease

1/100 Caucasians are heteroxygous for C2 deficiency and 1/10,000 are complemently deficient. Gene encoding C2 is in MHC. HLA-b18 and HLA-DR2 are tightly linked to C2 defiency in 90% of affected Cauacasiand who carry the same mutations in the C2 genes. The genes for C4 are also in the MHC. C4A and C4B genes are present in variable numbers of copies in the population. 30% of humans fail to express the products of 1, 2, or 3 of these genes and thus may have a lower C4 titer.

C3 deficiency causes increased susceptibility to all pyogenic infections. Congenital deficiencies of components of lectin pathway have been decribed including deficiency of mannose binding lectin (5% of population) and is associated with recurrent infections. Deficiency of MBL associated serine protease 2 (MASP2) is rare and may cause increased risk of infections of autoimmunity.

**Case 38: Mixed Essential Cryoglobulinemia**

**Summary:** Extent and duration of antigenic stimulation is the major determinant of the Ig levels in blood and body fluids. Patients with chronic infections have high levels (hypergammaglobulinemia). There are many chronic infectious diseases in which infections persists because of failure of the immune response to eliminate the causative agent (malaria, leishmaniasis, hepatitis). Other (mostly viral) infections in which the immune system fails to clearthe infection because of relative invisibility of the ingectious agent to the immune system include HSV and hep C.

Persistent immune response to Ag can lead to the formation of ICs that may be antigenic themselves. IgM antibodies can be formed to the IgG in the IC and are called rheumatoid factor.

Serum proteins and Igs in the immune complees may preceipitate in the cold (<37C) and then dissolve back into solution with rewarming. These proteins are called cryoglobulins and aren’t present (or are very difficult to detect) in normal blood. The detection of cryoglobulins in the blood (cryoglobulinemia) is associated with the deposition of ICs in the kidneys, small BVs, and other organs and can cause fatal IC disease. It is associated with aching joints, fatigue, and purpura, espeiclaly in patients with liver disease due to chronic hep C infection.

When circulating cryoglobulin complexes are deposited in the walls of BVs, they activate complement. The release of C5a chemoattractants causes leukoctes to infiltrate the BV wall, resulting in vasculities. The inflammation causes small BVs to burst and release blood into the skin, causing purpura. If the complexes are deposited in the synovia of the joints, arthritis occurs. Vasculitis in a critical area such as the brain or intestine is rare but can result in fatal bleeding.

Some monoclonal Igs produced by maligant cells in lymphoproliferative disorders such as multiple myeloma (IgG) or Waldenstrom’s macroglobulinemia (IgM) may also behave as cryoglobulins and precipitate in the cold (category I, 10-15% of patients).

**Case:** 42 yr old man with chronic fatigue, aching joints for months, raised red spots on his shins (purpuric spots caused b blood leaking from damaged vessels), liver was enlarged and palpable, very high alanine aminotransferase, other labs were normal, blood transfusion 20 yrs ago, high titer of AB against HCV and serum contained large amounts of HCV RNA determined by PCR, test for blood cryoglobulins was also positive (plasma refrigerated overnight and then placed in a tube that is used to measure hematocrit, centrigue in the cold and determine amount of cryoprecipitate), urine sample contained 4+ protein and innumerable RBCs inducating damage due to ICs in the kidney glomeruli, admitted with fever and severe abdominal pain and vomiting blood to ER, arterial perforation in the SI and intestinal contents had leadked from the perforation into the peritoneum causing peritonitis, on micro exam of gut wall tissue, intestinal BVs revealed inflammation (vasculitis) with deposition of ICs in the vessel walls

**Treatment:** goal for symptomatic cryoglobulinemia associated with HCV is to eradicate the HCV or at least reduce the level of viral infection (IFNa treatment, may prevent new infection of liver cells, may favor a Th1 response, use with ribavirin guanosine analog), for severe systemic illness such as intestinal vasculitis plasmapheresis is an option to rapidly lower the circulating titer of autoantibodies and ICs (withdraw blood from the vein, separate the plasma from the blood cells by centrigue or membrane separation, and reinfusion the patients blood cells resuspeded in donor plasma or another replacement solution)

**Explain that cryoglobulins represent serum immunoglobulin complexes that precipitate in the cold (<37°C). Explain that these complexes are absent (or virtually absent) in normal blood.**

**Describe cryoglobulinemia as a condition associated with immune complex deposition in the kidneys, blood vessels, or other organs which can lead to fatal disease.**

**Describe how deposition of immune complexes causes vasculitis.**

**Explain that ~40% of patients with chronic hepatitis C disease develop cryoglobulinemia, which is caused by production of both polyclonal IgG and monoclonal/polyclonal IgM rheumatoid factors. These rheumatoid factors bind the Fc of IgG (especially IgG molecules that have already bound antigen), thus promoting complex formation.**

Category II: polyclonal IgG and monoclonal IgM RF, 35-40% of patients

Category III: polyclonal IgG and polyclonal IgM RF, 50% of paitents (also see in autoimmune disorders such as SLE, erythematosus, Sjogren’s)

**Explain why reduction of circulation B cells (i.e. Rituximab treatment) may be beneficial for treatment of cryoglobulinemia and what the disadvantages are.**

Chimeric anti-CD20 monoclonal Ab consisting of human Ig1 k chain constant regions and murine variable regions. CD20 expressed constitituvely on B cells. When mab binds it leads to very efficient depletion of circulating B cells by complement fixation (MAC formation) and ADCC. Used in B cell lymphoma patients, autoimmune hemolytic anemia, and cold agglutinin disease. In HCV patients, it may help via B cell depletion leads to decreased Ab production and decreased in levels of circulating ICs. Because B cells are also important in acting as APCs and cytokine producing cells, removal of B cells may have addition benefits in symptomatic cryoglobulinemia patients.

Major concern is risk of infections. Possible that a decreased in anti-HCV Ab levels after B cell depletion may lead to uncontrolled replication of HCV

**Case 41: Autoimmune hemolytic anemia**

**Summary:** Various ways in which an infection can induce autoimmunity: disruption of tissue barrier to expose normally sequestered autoantigens, infectin microbe may act as adjuvant, microbe Ag may bind to self proteins and act as a hapten, microbe might infect self reactive lymphocytes (EBV) and induce autoantibody production, microbe might share cross reactive Ag with host (molecular mimicry). The MHC Ag carried by an individual may also confer susceptibility to or protection against certain autoimmune disease that can be incited by infection.

There are a few conditions in which an infection has been identified as the direct cause of the autoimmine disease. Rheumatic fever (polyarthritis, carditis), via s. pyogenes is an example of a type III autoimmune disease caused by IC formation. In renal glomeruli it is called post-streptococcal acute glomerulonephritis. Certain strep strains are nephrogenic. 3-4% of kids with a nephrogenic strain will develop acte glomerulonephritis within 1-2 weeks of onset of infection.

Autoimmune hemolytic anemia is associated with pneumonia caused by mycoplasma pneumonia. About 30% of paitents with this infection develop a transient increase in serum Ab to a RBC Ag and a small proportion of these people develop transient hemolytic anemia (Type II autoimmune disease) that persists as long as the infection persists. The decreased in RBC number (anemia) results from their destruction (hemolysis) as result of the binding of IgM autoantibody to a carb Ag on the RBC surface. When the infection subsides either spontaneously or with treatment, the autoimmune disorder disappears.

The autoantibodies in autoimmune hemolytic anemia are IgM or IgG. The IgM autoantibody agglutinates RBCs at temperatures below 37C. The anitbodies are therefore called cold hemagglutinins or cold agglutinins and the hemolytic disorder is known as cold agglutinin disease. Low titers of cold agglutinins occur in healthy patients. About 1/3 of patients with mycoplasma infection see a transient increase in titer without symptoms. They bring about hemolysis of RBCs either by complement fixation (IgM autoantibodies and complement, cleared by macrophages with CR1 and CR3) or adherence of the RBCs to the Fcg receptors (IgG autoantibodies) on cells of the fixed mononuclear phagocytic system of the spleen (and liver, other organs). The binding of certain rare autoantibodies that fi complement very efficiently causes formation of MAC on RBCs leading to intravascular hemolysis.

Transient autoantibodies most frequently develop after nonbacterial infections such as mono, mycoplasma infection. In mycoplasma, the cold autoantibodies react with a RBC surface antigen called I Ag. This is a branched carbohydrate structure that occurs on glycoporteins and glycolipids. I antigen isn’t fully expressed on RBCs until 6 months of age. Exactly how mycoplasma triggers transient production of RBC autoantibody isn’t known, although there is a strong biochemical similaritiy between the attachment site (ligand) for the mycoplasma on the host ell surface and the RBC Ag to which the autoantibody binds.

We must develop B cell tolerance to the I Ag in infancy. There is almost no TCR for this carb ag, so the help to move the B cells into follciles wouldn’t occur and specific B cells would undergo apoptosis n the T cell zone. Also, the B cells might be rendered anergic by soluble Ag or undergo apoptosis via Fas/FasL interaction. With mycoplasma infection, the disorder has orgin in the interaction of the infective agent with carb attachment sites on host cells. The mycoplasma adheres to the ciliated bronchial epithelium and also to RBCs and other cells via ligands that consiste of long carb chains of I ag that are capped with dialic acid. When bound to the carb chain that contains the I Ag sequence, mycoplasma may act as a carrier and the I Ag may act as a hapten.

The autoantibodies in the transient cold agglutinin syndrome resemble those in chronic cold agglutinin disease (chronic autoimmune disorder). This disorder runs a protracted course in which the autoantibodies are characteristically olgioclonal and lymphoproliferative disorders commonly develop. The autoantibodies against RBCs tend to be persistent. Thus, chronic cold agglutinin disease behaves as a variant of an IgM gammopathy (abnormal monoclonal or oligoclonal spike in immunoglobular electrophoretic pattern) called Waldenstrom’s macroglobulinemia.

Other anti-RBC antibodies include some that bind to RBCs at 37C (warm autoantibodies). Some syphilis patients have Donath-Landsteiner antibodies that bind to RBCs at cold temepratures but then cause lysis of RBCs via complement when the blood is warmed to 37C.

Autoimmune hemolytic anemia can also be induced by certain chemicals and drug.

**Case:** 34 yr old female with feverish cough and symptoms that have progressively worsened over the past few days, extremely pale, palms were compleletly white (healthy is reddish pink color when Hb is over 10g), fever, increased RR, scattered rhonchi (harsh breath sounds) at bases of both lungs, hematocrit and Hb levels were low, WBC count was elevated with increased absolute neutrophil count, CXR showed patchy infiltrates in both lower lungs (atypical pneumonia), sputum sample was negative for bacteria but showed abundant neutrophils, blood sample tested for presence of antibodies against RBCs (in presence of sodium citrate as an anticoagulation, the sample was chilled at 4C and the red cells clumped and the blood looked as though it had clotted, when warmed to 37C the RBCs dispersed, reversibly agglutinated in the cold), negative direct and indirect Coombs tests (test for presence of anti-RBC IgG), red cells were strongly agglutinated by Ab against C3, plasma was incubated in refridgerator with type 0 RBCs from normal blood and the red cells were strongly agglutinated but when tests with RBCs from umbilical cord the agglutination was weaker (I Ag not fully expressed in these cells)

**Treatment:** erythromycin for the pneumonia, for severe hemolysis use plasmapheresis (remove blood and centrifuge at 37C, resuspend cells in normal plasma and infuse into patient, removes IgM because 70% of IgM is in the plasma compartment, only 50% of IgG is in the plasma compartment)

**Describe the association of HLA-B27 with autoimmune arthritis occurring after infection by both *Chlamydia trachomatis* (Reiter’s) and a variety of enteric pathogens (leading to reactive arthritis).**

HLA-B27 associated with reactive arthritis after infection with c. trachomatis (Reiter’s syndrome), shigella flexneri, salmonella typhimurium and enteritidis, yersiia enterocolitica, and campylobacter jejuni

**Explain that certain cases of chronic arthritis following Lyme disease (i.e. those that do not respond to subsequent antibiotic therapy) is strongly associated with particular HLA-DR alleles.**

Chronic arthritis in Lyme disease via borrelia burgdorferi associated with HLA DR2 and DR4

**Explain that mycoplasmal pneumonia (also known as atypical pneumonia) is associated with patchy infiltrates in the lung (as viewed by X-ray), whereas complete opacity in one or more lobes is typical for pneumococcal pneumonia and most other bacterial pneumonias.**

**Describe that one result of infection with *Mycoplasma pneumoniae* can be the induction of an IgM autoantibody reactive with a carbohydrate (the I antigen) on the surface of red cells.**

IgA, IgD, and IgE don’t usually cause autoimmune hemolytic anemia because they don’t fix complement and there are no Fc receptors for these on macrophages (only have Fcg receptors). Fce receptors are expressed on mast cells and B cells and their engagement wouldn’t result in RBC destruction.

**Explain that the IgM autoantibody produced in mycoplasmal pneumonia binds fairly weakly (has low affinity) and agglutination is not seen unless the antibodies are mixed with cells in the cold (usually anti-coagulated blood that is chilled).**

**Recognize that paleness of the hands in warm conditions can be a sign of anemia.**

**Explain that “cold agglutinins” may bind sufficiently to red cells *in vivo* to promote complement deposition on those cells, leading to complement-mediated cell lysis (if the binding of complement is efficient) or red cell removal by phagocytic cells (via attachment of complement coated red cells to complement receptors on the phagocytes).**

**Explain why the antibody used in a direct “Coombs test” (designed to detect C3 or C4 on red cells) is aimed at either C3d or C4d, and not at other parts of C3 or C4 (question 3).**

In the direct and indirect Coombs tests, agglutination of Ab coated RBC is brought about by Ag against IgG. In the non-gamma Coobs test, an Ab against C3 or C4 is used to agglutinate RBCs coated with IgM that has fixed complement. The internal thiol ester that binds covalently to the IgM autoantibody is situated in the C3d and C4d fragments. The complement components C3b and C4b that are bound to autoantibody may be digested by fI to C3c + C3d and C4c + C4d. C3c and C4c would be released from the Ag:Ab complex and Ab against them wouldn’t agglutinate RBCs.